BD™ Stem Cell Enumeration Kit

For use on BD FACSCalibur™ and BD FACSCanto™ II systems

Features

- Enumerates total viable CD34+ cells and CD34+ cells as a percentage of total viable leucocytes
- Uses BD Trucount™ technology for an accurate and reproducible single platform assay[1,2]
- Consistent with International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines[1,2,5]
- Works with a wide variety of samples: normal and mobilized peripheral blood, fresh and post-thaw apheresis products, fresh and post-thaw bone marrow, and fresh and post-thaw cord blood specimens
- Supported by dedicated templates for the BD FACSCalibur™ system and automated software on the BD FACSCanto™ II system

The BD™ Stem Cell Enumeration Kit offers a single-tube, single-platform assay for accurate, reproducible, and rapid enumeration of CD34+ hematopoietic progenitor cells in a wide range of stem cell sources.

Transplantation of hematopoietic progenitor cells is used increasingly in the treatment of blood cell disorders, malignancies, and genetic abnormalities. Progenitor cells are rare and are found primarily in the bone marrow, with extremely low frequencies in peripheral blood. However, with the introduction of mobilization regimens (G-CSF, GM-CSF, and chemotherapy), peripheral blood has become the preferred source of stem cells.[2,3]

The CD34 antigen is present on immature hematopoietic precursor cells and hematopoietic colony-forming cells in bone marrow and blood, including unipotent and pluripotent progenitor cells.

An accurate measure of the CD34+ cell count is necessary for dose requirement protocols on stem cell transplantation.

The BD Stem Cell Enumeration assay simultaneously enumerates the total viable dual-positive (CD45+/CD34+) hematopoietic stem cells in absolute counts of CD34+ cells/µL and the percentage of viable leucocytes (CD45+) that are CD34-positive (CD34+).

The BD Stem Cell Enumeration Kit delivers accurate, reproducible, and precise results on both the BD FACSCalibur and the BD FACSCanto II systems.

The BD Stem Cell Enumeration Kit is sufficient for 50 tests and consists of:

- 50 BD Trucount tubes
- BD™ stem cell reagents: CD45 FITC/CD34 PE for the identification of leucocytes and hematopoietic stem precursor cells
- 7-Aminoactinomycin D (7-AAD) nucleic acid dye for viability measure
- Ammonium chloride lysing solution

Visit **bdbiosciences.com** for more information.

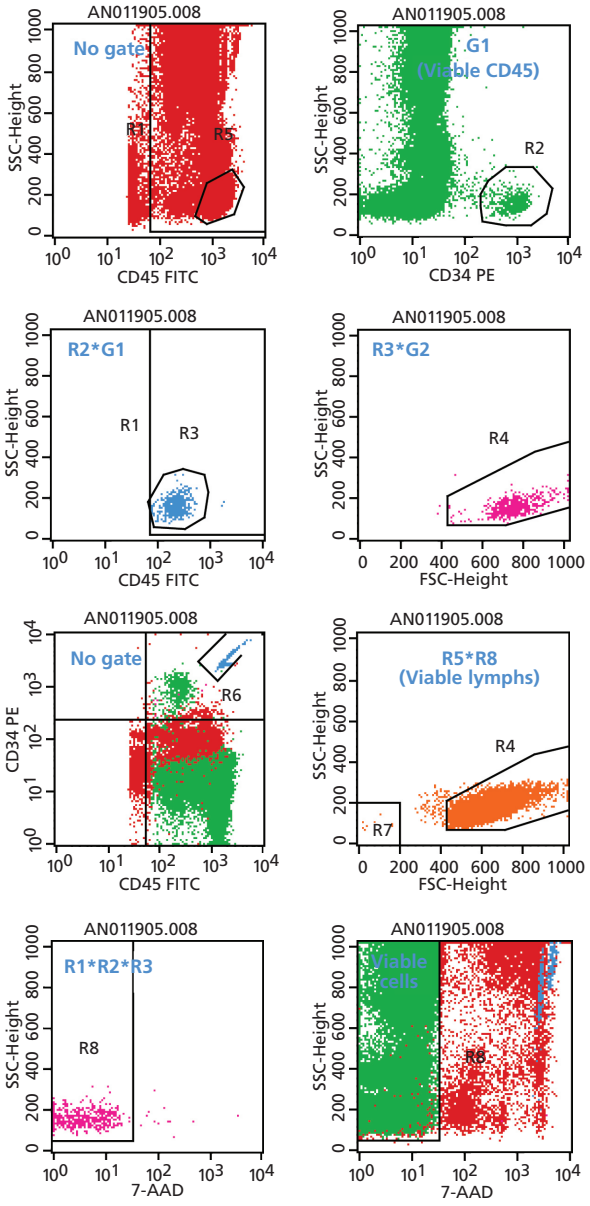
BD

BD Stem Cell Enumeration Kit on BD FACSCalibur

Proven consistency of BD CellQuest™ software

The BD Stem Cell Enumeration template for BD CellQuest™ v3.3 (Mac® OS9) and BD CellQuest™ Pro v4.0.2 (Mac® OS9), v5.2.1 (OS X®), or v6.0 (Intel®-based Mac Pro) on the BD FACSCalibur are available for the acquisition and analysis of samples processed with the BD Stem Cell Enumeration Kit.

Take advantage of the known methodology and consistency of BD CellQuest software and experience the ease of use of the incorporated regions, gates, statistics, and calculations consistent with ISHAGE recommendations.[1,2]



Enter These Values

Trucount = 49955.00

Dilution Factor = 1.00

Sample Volume = 100.00

File: AN011905.008
Sample ID: IQCB333E59011905R2
Patient ID: CORD BLOOD
TruCOUNT: 49955
Dilution Factor: 1
Custom Keyword #3:
Acquisition Date: 19-Jan-09
Total Events: 113660

Gate	Events	% Gated
Viable CD45	71418	79.54
G2	544	0.61
G3	541	0.60
Viable CD34	538	0.60
Beads	2913	3.24
Total CD34	549	0.61
Viable Lymphs	19251	21.44
TotalCD45	85079	94.75
Debris	0	0.00

Inspect All Dot Plots

Results

Viable CD34 = 92.26/µL

Viable CD45 = 12247.46/µL

Total CD34 = 94.15/µL

CD34 Viability = 98.00%

Viable CD34 of Viable CD45 = 0.75%

Figure 1. Example of result dot plots for a fresh cord blood specimen following analysis with BD CellQuest templates on a BD FACSCalibur.

A single-tube assay with BD Trucount technology
Pre-mixed antibodies are provided in optimized concentrations to reduce preparation time and to meet the productivity requirements of clinical labs.

The assay is designed to enumerate $CD34^+$ cells using a single BD Trucount tube which contains a known number of fluorescent beads, thus reducing the number of pipetting steps.[4]

Additionally, the assay has no need for isotype control, improving operational efficiency.[1,2,5]

Optimized for a wide range of samples
The BD Stem Cell Enumeration Kit supports a broad collection of specimens: normal and mobilized peripheral blood, fresh and post-thaw apheresis products, fresh and post-thaw bone marrow, and fresh and post-thaw cord blood specimens.

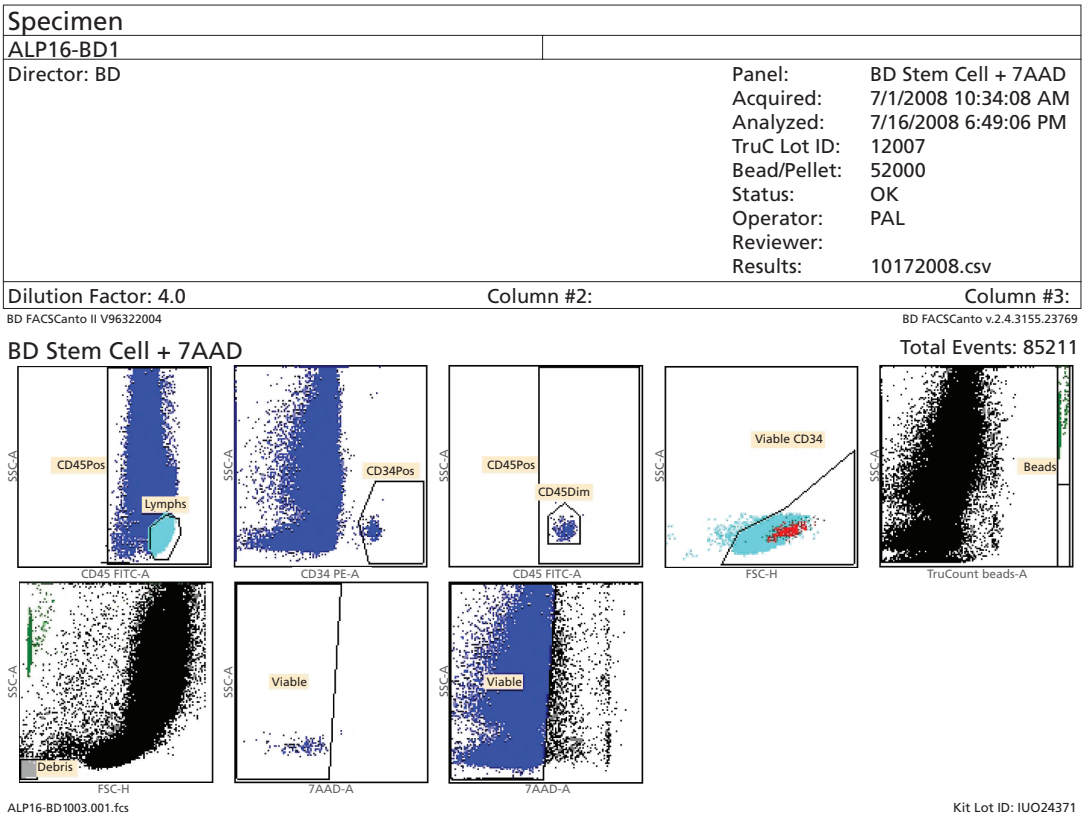
Demonstrated performance
Using BD FACSCanto II or BD FACSCalibur flow cytometers, the assay demonstrates equivalence to a predicate enumeration assay and linearity over a range from 0–1,000 cells/µL.

BD Stem Cell Enumeration Kit on BD FACSCanto II

Automatic gating with BD FACSCanto™ Clinical Software

A BD Stem Cell Enumeration module for BD FACSCanto™ clinical software v2.4 on BD FACSCanto II systems is available for acquisition and analysis of samples processed with the BD Stem Cell Enumeration Kit.

The BD Stem Cell Enumeration module for BD FACSCanto clinical software v2.4 offers automatic gating, calculations, and reporting, and is in-line with the ISHAGE protocol.[1,2]



CD34+ Viable Abs Cnt (cells/mL)	481.10
CD45+ Viable Abs Cnt (cells/mL)	123837.80
CD34+ Viable Events	303
CD45+ Viable Events	77994
Bead Events	1310
Viable CD34+ % Viable CD45+	0.39
CD34 Viability (%)	99.34
Viable CD34+ CV (%)	5.74

QC Messages
Inspect all dot plots.

Comments

Figure 2. Example of result dot plots for a fresh cord blood specimen following acquisition and analysis with the BD Stem Cell Enumeration module in BD FACSCanto clinical software.

Ready-to-use quality controls

The BD Stem Cell Control Kit Bi-Level is available to monitor the performance of antibody staining, red blood cell (RBC) lysis, and instrument setup. Controls contain stabilized human leucocytes, erythrocytes, and peripheral blood CD34⁺ cells in preservative medium.[2]

An assay for in vitro diagnostic use

Combining the precision of BD Trucount absolute counting beads with ISHAGE-based protocol guidelines for highly standardized and accurate results, the kit is designed to meet the quality requirements of clinical laboratories.

BD Stem Cell Enumeration Kit

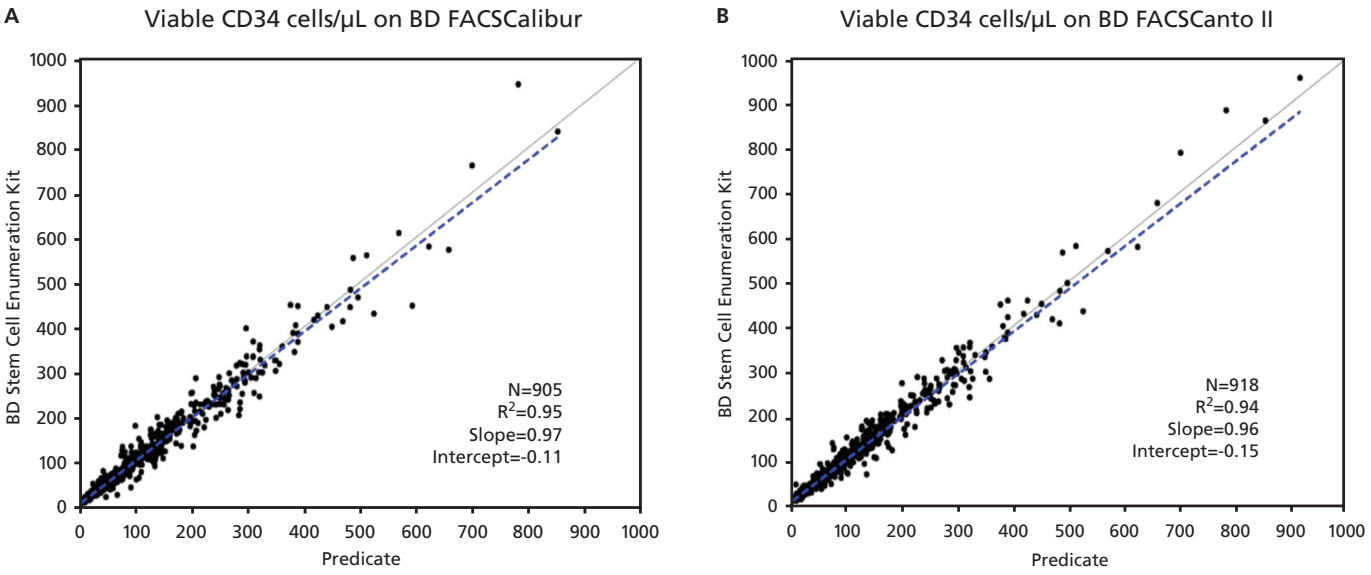


Figure 3. Regression between CD34+ counts derived using the BD Stem Cell Enumeration Kit and a predicate enumeration assay on a BD FACSCanto II **(A)** and a BD FACSCalibur **(B)**.

Table 1. Total system precision of CD34+ absolute counts on a BD FACSCalibur flow cytometer.

Control	Mean (cells/µL)	SD	%CV
High	35.3	1.83	5.2
Low	8.8	0.95	10.8

Table 2. Total system precision of CD34+ absolute counts on a BD FACSCanto II flow cytometer.

Control	Mean (cells/µL)	SD	%CV
High	35.3	2.02	5.7
Low	8.8	0.82	9.3

Ordering Information

Description	Size	Cat. No.
BD Stem Cell Enumeration Kit 50 tests Shipped with - SCE Template CD-ROM for BD CellQuest v3.3 or BD CellQuest Pro v4.0.2, v5.2.1, and v6.0 software - SCE BD FACSCanto application module CD-ROM for BD FACSCanto clinical software v2.4	50 tests	**344563**
BD Stem Cell Control Kit (Bi-Level)	2 mL per level	**340991**

Note: The BD Stem Cell Enumeration software module is designed for use on the BD FACSCanto II with the BD FACSCanto clinical software version 2.4. See the ordering information below for upgrading BD FACSCanto clinical software version 2.2 to version 2.4.

Description	Cat. No.
BD FACSCanto clinical software upgrade (v2.2 to v2.4) for the BD FACSCanto II system	**646603**

References

1. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. *J Hematother*. 1996;5:213-226.
2. Barnett D, Janossy G, Lubenko A, Matutes E, Newland A, Reilly JT. Guideline for the flow cytometric enumeration of CD34+ haematopoietic stem cells. Prepared by the CD34+ haematopoietic stem cell working party. General Haematology Task Force of the British Committee for Standards in Haematology. *Clin Lab Haematol*. 1999;21:301-308.
3. Gratwohl A, Passweg J, Baldomero H, Hermans J, Urbano-Ispizua A. Hematopoietic stem cell transplantation in Europe 1998. *Hematol J*. 2000;1:333-350.
4. Keeney M, Chin-Yee I, Weir K, Popma J, Nayar R, Sutherland DR. Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE guidelines. International Society of Hematotherapy and Graft Engineering. *Cytometry*. 1998;34:61-70.
5. Keeney M, Gratama JW, Chin-Yee IH, Sutherland DR. Isotype controls in the analysis of lymphocytes and CD34+ stem and progenitor cells by flow cytometry—time to let go! *Cytometry*. 1998;34:280-283.

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23-13829-01

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